H I L G A R D I A

A Journal of Agricultural Science Published by the California Agricultural Experiment Station

VOL. 17 OCTOBER, 1947 No. 17

LOCATION OF CURLY-TOP VIRUS IN THE BEET LEAFHOPPER, EUTETTIX TENELLUS[1]

HENRY H. P. SEVERIN[2]

INTRODUCTION

THE CURLY-TOP VIRUS is sometimes transmitted by the beet leafhopper, *Eutettix tenellus* (Baker), many days after the insect has had an opportunity to acquire the virus by feeding on an infected plant. The retention period of the virus in the insect has been reported to be as long as 151 to 180 days (Freitag, 1936).[3] The question of where the virus is during this time has interested a number of investigators.

Earlier attempts to determine the location of the virus in the leafhopper were failures. In experiments at this station (Severin, 1922), not a single case of curly top was obtained by inoculating healthy beets with various internal organs from infective beet leafhoppers dissected in physiological salt, Ringer's and Locke's solutions, and in juice expressed from healthy beets. The excrement from infective beet leafhoppers inoculated into the petioles of healthy beets also failed to produce curly top. Carsner and Stahl (1924) dipped the points of steel needles into drops of fresh excreta from infective beet leafhoppers and then pricked the excrement into two beet seedlings, but no disease resulted.

Later, however, Carter (1928*a*, *b*) succeeded in demonstrating curly-top virus in leafhopper saliva. He transmitted the virus by previously noninfective leafhoppers which had fed on a solution on which infective insects had previously fed, the incubation period of the disease in the beets in these instances being, as a rule, prolonged. An attempt (Severin, 1931) to demonstrate its presence in feces by a similar technique was unsuccessful: previously noninfective nymphs after feeding on a feeding solution containing the feces from infective beet leafhoppers failed to transmit curly top to sugar beets. But in 1938 Bennett and Wallace reported that a small amount of virus passes through the beet leafhopper and was present in active condition in

[1] Received for publication December 27, 1945.

[2] Entomologist in the Experiment Station.

[3] See "Literature Cited" for citations, referred to in the text by author and date.

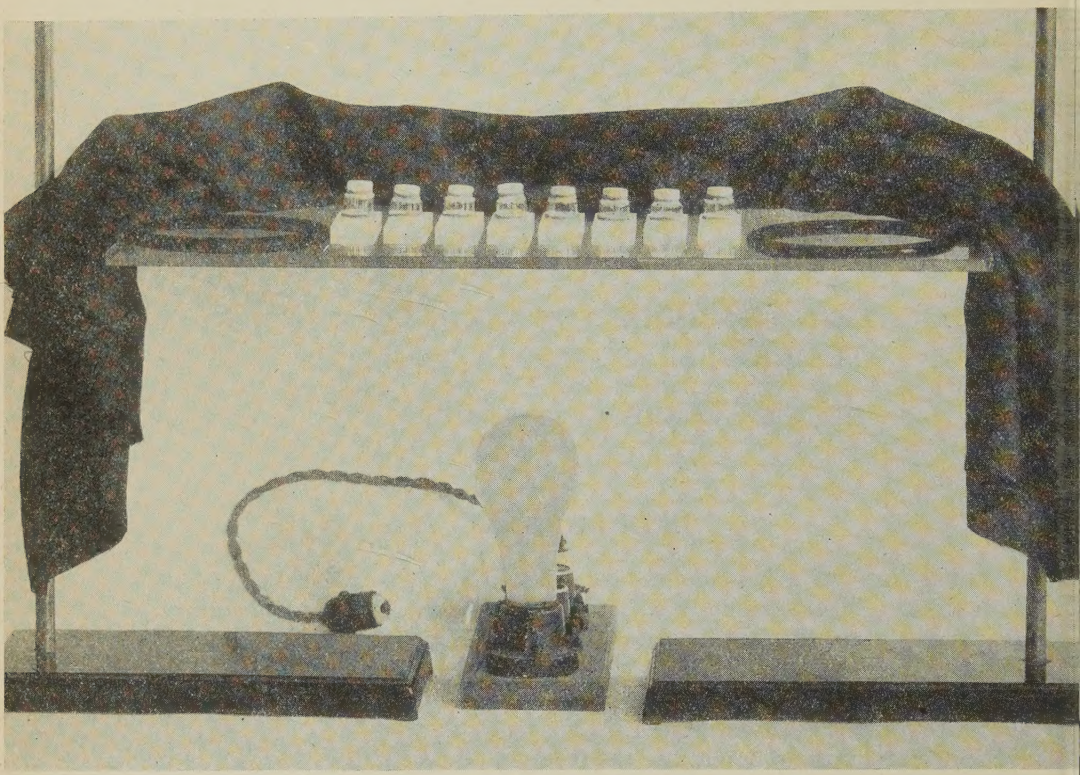


Fig. 1. Apparatus for drop method of feeding noninfective beet leafhopper nymphs on virus extract from internal organs. A drop of virus extract was placed on the "fishskin" covering the flared surface of the glass cage (see also fig. 2). The membrane with a drop of virus extract was placed over the depression of a microculture slide containing a small quantity of water. The feeding apparatus was placed on a glass plate above an electric incandescent lamp to attract the nymphs to the lighted membrane in the glass cage, and the top of the cages was covered with black sateen.

the feces. They also reported that virus was obtained from the blood, salivary glands, and alimentary canal of the beet leafhopper and concluded that the blood is the chief reservoir of the virus.

Dobroscky (1929) in her study on the aster-yellows virus in its insect vector, *Macrosteles divisus* (Uhler), suggested that "if certain portions of virus-carrying individuals were isolated and healthy insects made to feed on them, one might get an indication of the whereabouts of the virus."

This paper reports the results of further experiments to determine the location of the virus in the beet leafhopper. Attempts were made to determine whether the virus was located in the alimentary canal, salivary glands, saliva, blood, and ovaries of infective beet leafhoppers. Noninfective leafhoppers were fed on filtered solutions in which internal organs of infective insects had been crushed. Investigations were also undertaken on the filterability of the curly-top virus in the various internal organs of infective leafhoppers.

METHODS

Feeding Solution. A large number of experiments were conducted with various feeding solutions using different kinds and percentages of sugar. The solutions used were juices from the leaves, petioles, leaves and petioles, beet root, or the entire beet plant, often mixed with various amounts of different sugar solutions.

In our early work the filtrates prepared from the internal organs of infective beet leafhoopers were often incubated, sometimes for a period of

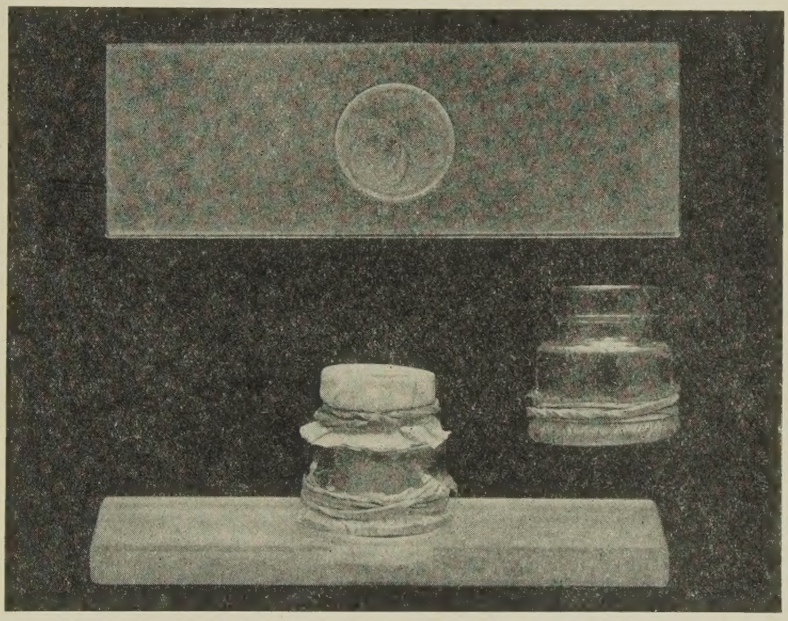


Fig. 2. Microculture slide and glass cage used in feeding noninfective nymphs (shown on glass plate in fig. 1).

1 to 7 days. It is not necessary to enter into a discussion of these experiments, which usually gave negative results, since many other experiments have demonstrated that the curly-top virus is inactivated by prolonged incubation of aerobic filtrates.

In our later work the feeding solution mostly used was as follows:

6 cc filtered healthy beet-root juice
1 cc soluble starch 2 per cent
1 cc beet sugar 2 per cent

Stahl and Carsner (1918) were the first to report a method of obtaining beet leafhoppers noninfective as to curly top. During the process of hatching from a diseased beet, the nymphs before feeding were transferred with a camel's-hair brush to a healthy beet, and the leafhoppers failed to produce the disease. Successive generations have been reared on healthy beets during

a period of thirty years, and the leafhoppers remained noninfective throughout this period.

Dissections. Before dissecting out the internal organs from infective beet leafhoppers, the insects were transferred from diseased beets to an empty cage and were kept without food overnight. This was done to avoid the possible transmission of unchanged diseased beet juice in the alimentary canal. The adults were dropped into a small phial containing sterile distilled water, and the bottle was shaken so that the wings became wet and they were unable to fly. The leafhoppers were transferred to fresh distilled water in a Syracuse watch glass with the bottom covered with a mixture of paraffin and beeswax. While the insect was submerged in the distilled water the internal organs were dissected from the adults with a small triangular or spear-shaped scalpel. The internal organs were thoroughly washed with many changes of sterile, distilled water by agitating the liquid with a medicine dropper. The internal organs were transferred to the feeding solution and were crushed with a small pestle in a watch glass.

Filtration. The feeding solution was filtered through coarse and fine Berkefeld candles. Except as otherwise noted, after the internal organs were crushed in the solution each solution was again filtered through a small fine Berkefeld candle.

Feeding Leafhoppers on Virus Extracts. Carter (1927, 1928*a*) perfected methods of feeding nymphs and adult beet leafhoppers on liquids. Using this feeding apparatus in a modified form, Severin and Swezy (1928) demonstrated, from both diseased sugar beets and crushed infective beet leafhoppers, the filterability of the curly-top virus. These feeding equipments required a considerable amount of liquid and it was assumed that the dilution magnitude of the curly-top virus would play an important role in this work. Hence a feeding technique was devised in which a small quantity of solution was employed in a microculture slide as described in a previous paper (Severin and Swezy, 1928). In some of the experiments noninfective nymphs were fed on a drop of solution placed on the "fishskin" covering the flared surface of a glass cage. The membrane with the drop of liquid was placed over the depression of a microculture slide containing a small quantity of water to prevent evaporation. The feeding apparatus was placed on a glass plate above an electric incandescent lamp (figs. 1 and 2) and the top of the glass cages was covered with black sateen. The nymphs, which had been fasted for a few hours, were attracted to the lighted membrane and soon fed on the drop of solution.

LOCATION OF VIRUS IN BEET LEAFHOPPER

Midintestine, or Stomach. Experiments were performed to determine whether the curly-top virus was located in the midintestine, or stomach, of infective beet leafhoppers. The fore and hind intestine were torn from the alimentary canal in the dissections and the midintestine left intact. The number of stomachs per cubic centimeter of solution usually varied from 10 to 25, but sometimes as many as 60 were used. The crushed stomachs in the solution were either fed directly to previously noninfective nymphs or after filtering through a fine Berkefeld candle. The results of inoculating healthy

sugar-beet seedlings with curly-top virus by previously noninfective nymphs fed on solution containing crushed midintestines from infective adults was as follows:

	Beets inoculated	Beets infected
Unfiltered solutions	204	8
Filtered solutions	240	9

Salivary Glands. A large number of experiments were conducted with different feeding solutions containing crushed salivary glands dissected from infective beet leafhoppers to determine whether the curly-top virus was located in these organs. In dissecting the adults, the head was severed from the thorax in sterile distilled water. If the heads were allowed to remain in the water for a few minutes the salivary glands protruded from the opening and could easily be dissected out with a small triangular scalpel. The number of salivary glands per cubic centimeter of solution varied from 15 to 25. Healthy sugar-beet seedlings were inoculated with curly-top virus by means of previously noninfective nymphs fed on unfiltered solutions containing these crushed salivary glands. Of the 352 beets thus inoculated, 39 developed curly-top.

TABLE 1

RESULTS OF INOCULATING HEALTHY SUGAR-BEET SEEDLINGS WITH PREVIOUSLY NONINFECTIVE BEET LEAFHOPPERS FED ON FEEDING SOLUTIONS INTO WHICH INFECTIVE LEAFHOPPERS HAD INJECTED SALIVA

Feeding solution	Infective nymphs fed feeding solution	Beets inoculated	Beets infected	Incubation period of disease in plant
	number	*number*	*number*	*days*
8 cc steam-extracted beet-root juice plus 8 cc 5 per cent beet-sugar solution	200–500	40	3	12, 13, 16
16 cc healthy beet-root juice	200–500	18	7	9, 12, 13, 14, 14, 14, 36
16 cc filtered healthy beet-root juice	200–500	18	0	.....

Saliva. Experiments on the transmission of curly-top virus by previously noninfective beet leafhoppers which had fed on solutions in which saliva was injected by infective leafhoppers were made with three feeding solutions. The feeding equipment consisted of a small culture or "Esmarch" dish (50 x 10 mm) containing about 16 cc of solution (fig. 3) as described in a previous paper (Severin and Swezy, 1928). The results are indicated in table 1.

As in Carter's experiments, previously mentioned (p. 545), the incubation period of the disease in the beet was occasionally prolonged: in one beet it was 36 days, as compared with the usual period of 10 to 14 days under greenhouse conditions.

Blood. Attempts were made to determine whether the curly-top virus was located in the blood of infective beet leafhoppers. The aorta opens in the vicinity of the brain. Hence the leafhoppers were decapitated, and the blood was allowed to ooze into a drop of the culture medium. The solution consisted of sterile distilled water containing 5 per cent alcoholic pure sucrose. Blood from 25 infective beet leafhoppers was used for each cubic centimeter of solu-

tion. Previously noninfective nymphs were fed on the solution, both unfiltered and filtered, and then transferred to healthy beet seedlings. The results were as follows:

	Beets inoculated	Beets infected
Unfiltered solution	14	4
Filtered solution	50	1

Ovaries. Tests were made to determine whether the curly-top virus could be transmitted with the ovaries crushed in a solution. The blood, which contains the curly-top virus, bathes the ovaries; hence they were thoroughly

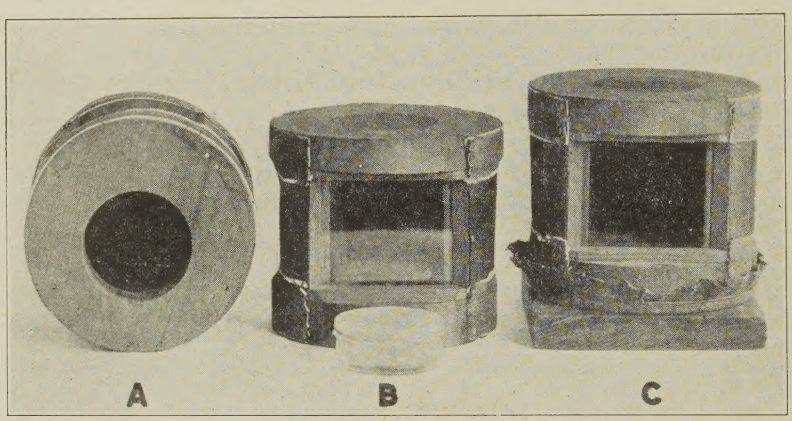


Fig. 3. Small culture or "Esmarch dish" containing feeding solution placed directly behind the glass in a cage lined with black sateen.

washed in many changes of sterile, distilled water. The ovaries from 10 to 50 females were dissected and crushed in 1 cubic centimeter of solution. The microculture slide and drop methods of feeding were used. Previously noninfective nymphs after feeding on the culture solution containing the crushed ovaries failed to transmit curly-top virus to any of 112 beets in 56 feeding experiments.

SUMMARY

Previously noninfective beet leafhoppers obtained the curly-top virus and transmitted the infective principle to healthy beet seedlings by feeding on solutions containing crushed stomach, salivary glands, saliva, and blood from infective leafhoppers and on the filtrates prepared with these organs. Negative results were obtained with the ovaries dissected from infective female leafhoppers.

The saliva containing the virus was injected into a feeding solution by infective beet leafhoppers and was recovered by previously noninfective insects and transferred to beet seedlings.

LITERATURE CITED

BENNETT, C. W., and H. E. WALLACE.
1938. Relation of the curly-top virus to the vector, *Eutettix tenellus,* Jour. Agr. Res. **56**:31–51.

CARSNER, E., and C. F. STAHL.
1924. Studies on curly-top disease of the sugar beet. Jour. Agr. Res. **28**:297–320.

CARTER, W.
1927. A technique for use with homopterous vectors of plant disease, with special reference to the sugar-beet leafhopper, *Eutettix tenellus* (Baker). Jour. Agr. Res. **34**:449–51.
1928*a*. An improvement in the technique for feeding homopterous insects. Phytopathology **18**:246–47.
1928*b*. Transmission of the virus of curly-top of sugar beets through different solutions. Phytopathology **18**:675–79.

DOBROSCKY, I. D.
1929. Is the aster-yellows virus detectable in its insect vector? Phytopathology **19**:1009–1015.

FREITAG, J. H.
1936. Negative evidence on multiplication of curly-top virus in the beet leafhopper, *Eutettix tenellus.* Hilgardia **10**(9):263–302.

SEVERIN, H. H. P.
1921. Minimum incubation periods of causative agent of curlyleaf in beet leafhopper and sugar beet. Phytopathology **11**:424–29.
1922. Curlyleaf transmission experiments with beet leafhopper. Jour. Econ. Entom. **15**:182.
1931. Modes of curly-top transmission by the beet leafhopper, *Eutettix tenellus* (Baker). Hilgardia **6**(8):253–76.

SEVERIN, H. H. P., and O. SWEZY.
1928. Filtration experiments of curly top of sugar beets. Phytopathology **18**:681–90.

STAHL, C. F., and E. CARSNER.
1918. Obtaining beet leafhoppers nonvirulent as to curly-top. Jour. Agr. Res. **14**:393–94.

SPINACH YELLOW DWARF

HENRY H. P. SEVERIN and DONALD H. LITTLE

SPINACH YELLOW DWARF[1]

HENRY H. P. SEVERIN[2] and DONALD H. LITTLE[3]

INTRODUCTION

FIVE VIRUS DISEASES of spinach (*Spinacia oleracea* L.) have thus far been reported to occur under natural conditions in the United States—namely, spinach blight (McClintock and Smith, 1918)[4], which is identical with common cucumber mosaic (Hoggan, 1933); aster yellows (Kunkel, 1926; Severin, 1934; Severin and Frazier, 1945); sugar-beet curly top (Severin and Henderson, 1928; Scott, 1935; Adams, 1936); beet mosaic (Jones, 1931; Hoggan, 1933; Smith, 1934); and spotted wilt (Gardner, Tompkins, and Thomas, 1937).

This paper deals with still another naturally occurring virus disease of spinach—spinach yellow dwarf. The aspects covered include symptoms and host range of the disease, the properties and aphid vector of the virus, transmission of the virus by single aphids, a comparison of the transmission of the virus by one species of aphid with mechanical inoculation, and retention of the virus by aphids.

MATERIALS AND METHODS

Source of Virus. The source of the spinach-yellow-dwarf virus was naturally infected spinach plants obtained from the truck-crop fields near San Pablo. The virus was retained through repeated mechanical inoculations and aphid transmission to spinach.

Spinach Extract. Juice from diseased spinach plants was obtained by grinding the plants to a pulp in a sterilized food chopper or mortar. The pulp was then placed between two layers of cheesecloth and the sap expressed by hand into a sterile glass dish.

Mechanical Inoculation. The method of mechanical inoculation used is that described by Rawlins and Tompkins (1936). After inoculation, the inoculum and the carborundum were washed from the leaves with water. The virus extract from each preparation was inoculated into 5 healthy spinach plants.

Variety of Spinach. Long Standing Bloomsdale spinach was used in studies of properties and aphid transmission of the virus and as a source of virus in host-range studies. This variety grows rapidly and remains in good condition a long time without bolting to seed.

Noninfective Aphids. Noninfective green peach aphids were obtained from populations reared on healthy sugar beets maintained in the greenhouse.

Methods of Transferring Aphids. High populations of aphids were transferred by cutting off leaves which bore large numbers of aphids, then placing the leaves on the inner or youngest leaves of another plant. Small

[1] Received for publication February 6, 1946.

[2] Entomologist in the Experiment Station.

[3] Formerly graduate student in Entomology and Parasitology.

[4] See "Literature Cited" for citations, referred to in the text by author and date.

numbers of aphids were transferred from plant to plant with a moistened camel's-hair brush.

SYMPTOMATOLOGY

The most conspicuous symptoms of the disease on naturally infected spinach are yellow blotches on the outer, or oldest, leaves, and dwarfed, puckered, curled, mottled inner, or youngest, leaves. Typical symptoms are illustrated in plate 1.

The first visible symptoms of the disease on spinach are a clearing of the veinlets (plate 2, *A*) and curvature of the midrib. These symptoms develop within from 12 to 14 days after inoculation in the greenhouse, and from 25 to 35 days out of doors during the winter.

The younger leaves next show yellow and green mottling, puckering, curling, and blisterlike elevations (plate 1). The older leaves show numerous small chlorotic areas (plate 2, *B*) which later coalesce and form conspicuous yellow blotches (plate 2, *C*). The yellow blotches become necrotic, the condition usually beginning at the basal margin of the leaf (plate 2, *D*) and progressing toward the tip. The heart of the infected plant is stunted, with shortened petioles on the younger leaves (plate 1).

As the disease progresses, the older leaves gradually become brown and dry. The young leaves usually remain mottled and distorted for a week or more after the older leaves are dead, but eventually they also become yellow and die.

HOST RANGE

Natural Infection. The natural host range of the yellow-dwarf virus so far known is limited to spinach. The virus was recovered from naturally infected spinach by inoculation of extracted sap into healthy spinach.

Experimental Infection. The following ten horticultural varieties of spinach have been experimentally infected with the virus by mechanical inoculation:

Chenopodiaceae:

Spinacia oleracea, varieties Broad Flanders, Giant Thick Leaved, Juliana, King of Denmark, Long Standing Bloomsdale, Prickly Seeded Dark Green, Savoy Leaved or Bloomsdale, Thick Leaved Nobel, Virginia Savoy, and Virofloy.

The variety Virginia Savoy spinach is resistant to spinach blight (Smith, 1920; Hoggan, 1933), but is susceptible to the yellow-dwarf virus.

Recovery of Virus. The virus was recovered from all the experimentally infected varieties of spinach by inoculation of extracted sap in healthy spinach.

Nonsusceptible Plants. The following economic and ornamental flowering plants, tested by mechanical inoculation, are nonsusceptible to the disease:

Aizoaceae:

Tetragonia expansa, New Zealand spinach

Campanulaceae:

Campanula medium, canterbury bells

Chenopodiaceae:

Beta vulgaris, varieties of garden beets: Crimson King, Crosby's Egyptian, Detroit Dark Red, Early Blood Turnip, Extra Early Flat Egyptian, Early Wonder, Ferry's Crosby, and Good for All

Beta vulgaris, varieties of mangel-wurzel or stock beets: Danish Red Giant Eckendorf, Danish Sludstrup, Danish Yellow Giant Eckendorf, Giant Half Sugar Green Top, Giant Half Sugar Rose Top, Giant Yellow Intermediate, Mammoth Long Red, Yellow Leviathan

Beta vulgaris var. *cicla*, varieties of Swiss chard: Large Ribbed Dark Green, Lucullus Dark Green

Compositae:

Callistephus chinensis, aster

Lactuca sativa, lettuce

Cruciferae:

Brassica oleracea var. *capitata*, cabbage

Brassica campestris, common yellow mustard

Mathiola incana var. *annua*, stock or gilly flower

Cucurbitaceae:

Cucurbita pepo, varieties of squash: Italian or Zucchini, White Bush Scallop

Citrullus vulgaris, varieties of watermelons: Kleckey's Sweet, Klondike

Cucumus sativus, White Spine cucumber

Geraniaceae:

Pelargonium zonale, geranium

Leguminosae:

Lupinus hartwegii

Malvaceae:

Gossypium hirsutum, cotton, Acala variety

Nyctaginaceae:

Mirabilis jalapa, four-o'clock

Primulaceae:

Primula veris, cowslip

Solanaceae:

Capsicum frutescens, California Wonder pepper

Lycopersicon esculentum, Marglobe tomato

Nicotiana glutinosa

Nicotiana alata var. *grandiflora*, tuberous-flowered tobacco

Nicotiana tabacum, Turkish tobacco

Petunia hybrida, petunia, Rosy Morn variety

Datura stramonium, stramonium

Solanum tuberosum, potato

Umbelliferae:

Foeniculum vulgare var. *dulce*, Florence fennel

Apium graveolens var. *dulce*, celery, Golden Self-Blanching variety

The host range of the virus so far discovered is thus limited to varieties of spinach. Plants in only thirteen families were tested, however, and it may be possible that economic or ornamental plants or weeds in other families are susceptible. A limited host range for this virus would not be suprising, for those of other mosaic viruses are limited. For example, Tompkins and Middleton (1941) found that the host range of a mosaic disease of *Primula obconica* is limited to two additional species within the same genus, namely, *P. malacoides* and *P. sinensis*.

PROPERTIES OF THE VIRUS

Thermal Inactivation. The thermal inactivation of the spinach-yellow-dwarf virus was determined in the extracted sap from leaves of experimentally infected spinach. Ten cubic centimeters of diseased sap was poured into thin glass test tubes, which were plugged with cotton and submerged in a water bath maintained at the desired temperature by an electric thermostat. An

agitator connected to an electric motor kept the water in circulation to maintain an evenly distributed temperature. The time of exposure in the water bath was 11 minutes, 1 minute being allowed for lag. After exposure at the desired temperature, the extract was cooled rapidly by partly submerging the test tubes in running tap water. Unheated controls were used in each test. Determinations were made at 5° C intervals. The results were as follows:

Temperature, ° C	Plants inoculated	Per cent infected
Unheated control	25	84
40	25	84
45	15	80
50	25	4
55	15	0
60	25	0
65	25	0
70	25	0

These data show that the virus remained active at 45° and that 1 infection was obtained from 25 inoculations at 50°; but no infections occurred at 55° to 70°.

Effect of Freezing Virus Extract. Two extractions of sap from leaves of experimentally infected spinach plants were placed in cold storage at –18° C immediately after extraction. Monthly inoculations of the virus extract in healthy spinach were made for a period of 6 months. The results were as follows:

Age of virus extract, months	Plants inoculated	Plants infected
Control	10	9
1	10	8
2	10	9
3	10	6
4	10	5
5	10	4
6	10	1

Thus freezing did not inactivate the virus at the end of 6 months, although only 1 infection was obtained with 10 plants inoculated at the end of this time.

Tolerance to Aging in Vitro. Tests were made to determine the inactivation of the virus in diseased spinach sap exposed to the air at room temperatures. Five cc of the expressed juice from experimentally infected spinach plants was poured into sterile test tubes which were plugged with cotton. Inoculations of the virus extract stored in test tubes were made at intervals of 24 hours for a period of 9 days. The following data were obtained, 25 plants being inoculated in each test:

Days exposed	Plants infected	Days exposed	Plants infected
0; Control	19	5	2
1	15	6	3
2	14	7	2
3	7	8	0
4	7	9	0

These data indicate that infectivity of the virus occurred after the extract was aged *in vitro* from 3 to 7 days and that no infections were obtained at the end of 8 and 9 days.

Tolerance to Dilution. In determining the tolerance of the virus to dilution, the juice expressed from experimentally infected spinach was diluted with sterile distilled water. The infections obtained were as follows:

Dilution	Plants inoculated	Per cent infected
Undiluted control	45	80
1:10	45	87
1:100	45	76
1:1,000	45	36
1:5,000	45	16
1:10,000	45	13
1:15,000	35	3
1:20,000	35	6
1:25,000	35	0
1:50,000	45	0

The tolerance to dilution was 1:20,000. There is considerable variation in the tolerance to dilution of the virus with different extractions; with three virus extracts, infections were obtained at dilutions of only 1:100.

APHID TRANSMISSION OF VIRUS

By Green Peach Aphid. Large populations of the green peach aphid, *Myzus persicae* (Sulzer) have been observed in the fields near San Pablo. This aphid transmitted the spinach-yellow-dwarf virus from experimentally infected to healthy spinach plants in the greenhouse.

By Single Aphids during Short Feeding Time. An experiment was conducted to determine whether single green peach aphids were able to transmit the virus during short feeding periods. Single previously noninfective wingless aphids were fed 5 minutes on a diseased plant and then 5 minutes on a healthy spinach plant. Two infections were obtained with 25 aphids tested.

Comparison of Transmission of Virus by Aphids with Mechanical Inoculation. Transmission of the virus by 20 green peach aphids was compared with that by mechanical inoculation. After a large population of previously noninfective green peach aphids was reared on experimentally infected spinach plants, lots of 20 aphids were transferred from diseased plants to each of 5 healthy spinach plants. The extract from each diseased plant on which the aphids fed was also inoculated into 5 healthy spinach plants. The results were as follows:

Test no.	Plants infected by aphid transmission	Plants infected by mechanical inoculation
1	4	5
2	4	3
3	2	4
4	0	4
5	0	3
Total	10	19

Infections obtained by aphids thus averaged 40 per cent and by mechanical inoculation 76 per cent.

Retention of the Virus. Two experiments were conducted to determine the length of time the green peach aphids would retain the virus. In the first experiment, 5 lots of 20 wingless green peach aphids were fed upon diseased spinach plants for a period of 3 days, and then each lot was transferred daily to 3 successive healthy spinach plants. The aphids remained on the third plant for a period of 1 week. The results were as follows, the plus sign indicating production of the disease and the minus sign indicating that no disease resulted:

Test no.	1st day	2d day	3d day
1	+	−	−
2	+	−	−
3	+	−	−
4	+	−	−
5	+	−	−
Total +	5	0	0
Total −	0	5	5

Thus aphids infected only those plants fed upon the first day.

In the second experiment, 5 lots of 20 wingless aphids were fed upon diseased spinach plants for a period of 3 days, and then each lot was transferred hourly to 10 successive healthy spinach plants. As appears in table 1, 5 lots of aphids infected a spinach plant during the first hour, and 2 lots during the second hour also, and none thereafter.

TABLE 1

RETENTION OF SPINACH-YELLOW-DWARF VIRUS BY LOTS OF 20 GREEN PEACH APHIDS, *Myzus persicae*, TRANSFERRED HOURLY TO 10 SUCCESSIVE HEALTHY SPINACH PLANTS

Test no.	Results* on successive plants, with hourly transfers									
	1st	2d	3d	4th	5th	6th	7th	8th	9th	10th
1	+	+	−	−	−	−	−	−	−	−
2	+	+	−	−	−	−	−	−	−	−
3	+	−	−	−	−	−	−	−	−	−
4	+	−	−	−	−	−	−	−	−	−
5	+	−	−	−	−	−	−	−	−	−
Total +	5	2	0	0	0	0	0	0	0	0
Total −	0	3	5	5	5	5	5	5	5	5

* The plus sign (+) indicates the production of the disease, and the minus sign (−) shows that no disease resulted.

DESCRIPTION OF SPINACH-YELLOW-DWARF VIRUS

Name: Spinach yellow dwarf.

Host plant: Spinach (*Spinacia oleracea*).

Symptoms of disease: Vein clearing, yellow and green mottling, puckering, curling, blisterlike elevation, curvature of midribs and petioles, and stunting of young leaves. Small chlorotic areas, which fuse, forming yellow blotches, followed by necrosis and drying of outer leaves. Yellowing and death of younger leaves.

Incubation period of disease: 25 to 30 days out of doors during winter.

Properties of virus: Thermal inactivation 55° C in 10-minute exposures, resistance to aging *in vitro* 8 days, tolerance to dilution 1:20,000.

Modes of transmission: Natural inoculation by green peach aphid, *Myzus persicae;* mechanical inoculation with expressed juice.

SUMMARY

The symptoms of yellow dwarf on naturally and experimentally infected spinach are described.

The host range of this virus is limited to spinach. Twenty-six species of plants in 23 genera belonging to 13 genera, were nonsusceptible to the virus. Some of the properties of the spinach-yellow-dwarf virus are summarized as follows: thermal inactivation of the virus was 55° C in 10-minute exposures. Freezing sap extracted from diseased spinach kept in cold storage at –18° C did not inactivate the virus at the end of 6 months. An inactivation of the virus occurred after the virus extract was exposed to the air at room temperature for a period of 8 days. The tolerance to dilution of extracted diseased spinach juice was 1:20,000.

The green peach aphid, *Myzus persicae* (Sulzer), was demonstrated to be a vector of the virus. Two of 25 previously noninfective wingless aphids transmitted the virus after feeding 5 minutes on a diseased plant and 5 minutes on each healthy plant. Infections obtained by lots of 20 aphids averaged 40 per cent, and by mechanical inoculation of the virus extract from each plant on which the aphids had fed, 76 per cent. Lots of 20 wingless aphids transmitted the virus from diseased to healthy plants during the first day, but failed to infect plants during the second day and during the following week. Five lots of 20 infective aphids, when transferred hourly to successive sets of healthy spinach for a period of 10 hours, infected 5 plants during the first hour, 2 plants during the second hour, and none thereafter.

LITERATURE CITED

ADAMS, J. F.
1936. The curly-top disease of spinach in south Texas. Assoc. Seed Growers' Inc. (Leaflet.)

GARDNER, M. W., C. M. TOMPKINS, and R. H. THOMAS.
1937. Factors affecting the prevalence of the spotted-wilt virus. [Abstract.] Phytopathology 27:129.

HOGGAN, I. A.
1933. Some viruses affecting spinach and certain aspects of insect transmission. Phytopathology 23:446–74.

JONES, L. K.
1931. The mosaic disease of beets. Washington Agr. Exp. Sta. Bul. 250:1–15.

KUNKEL, L. O.
1926. Studies on aster yellow. Amer. Jour. Bot. 13:646–705.

MCCLINTOCK, J. A., and L. B. SMITH.
1918. The true nature of spinach blight and the relation of insects to its transmission. Jour. Agr. Res. 14:1–60.

RAWLINS, T. E., and C. M. TOMPKINS.
1936. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopathology 26:578–87.

SCOTT, G. W.
1935. Spinach production in California. California Agr. Exp. Sta. Cir. 92:1–26.

SEVERIN, H. H. P.
1934. Transmission of California aster and celery-yellows virus by three species of leafhoppers. Hilgardia 8(10):339–61.

SEVERIN, H. H. P., and C. F. HENDERSON.
1928. Some host plants of curly-top. Hilgardia 3(13):339–84.

SEVERIN, H. H. P., and N. W. FRAZIER.
1945. California aster yellows on vegetable and seed crops. Hilgardia 16(12):573–96.

SEVERIN, H. H. P., and J. H. FREITAG.
1933. Some properties of the curly-top virus. Hilgardia 8(1):1–48.

SMITH, K. M.
1934. The mosaic disease of sugar beets and related plants. [Gt. Brit.] Min. Agr. and Fisheries Jour. 41:269–74.

SMITH, L. B.
1920. Breeding mosaic resistant spinach and notes on malnutrition. Virginia Truck Exp. Sta. Buls. 31–32:135–60.

TOMPKINS, C. M., and J. T. MIDDLETON.
1941. A mosaic disease of *Primula obconica* and its control. Jour. Agr. Res. 63:671–79.

PLATES

Plate 1.—King of Denmark spinach (*Spinacia oleracea*) experimentally infected with yellow-dwarf virus showing yellow blotches on the older, puckered, and curled leaves; and showing dwarfed, puckered, mottled, younger leaves, some displaying curved petioles.

Plate 2.—King of Denmark spinach (*Spinacia oleracea*) infected with yellow-dwarf virus: *A*, cleared veinlets on youngest leaf; *B*, leaf showing numerous, small, circular, chlorotic areas; *C*, yellow blotches formed by fusion of the chlorotic areas; *D*, chlorosis and necrosis of leaf.

WEEDS EXPERIMENTALLY INFECTED WITH BEET-MOSAIC VIRUS

HENRY H. P. SEVERIN and ROGER M. DRAKE

WEEDS EXPERIMENTALLY INFECTED WITH BEET-MOSAIC VIRUS[1]

HENRY H. P. SEVERIN[2] and ROGER M. DRAKE[3]

INTRODUCTION

IT IS IMPORTANT to know what plants growing in the cultivated areas and on the uncultivated plains and foothills are reservoirs of the beet-mosaic virus. After the pasture vegetation becomes dry on the plains and foothills, enormous flights of aphid vectors fly into the cultivated areas, and are often abundant on favorable weeds, varieties of beets, and other economic plants. Unpublished data indicate that the host range of the virus among economic plants is limited to plants belonging to the families of Azioaceae, Chenopodiaceae, and Papaveraceae.

This paper deals with the weed host range of the beet-mosaic virus. A study was made of the sequence of symptoms of experimentally infected weeds so that naturally infected plants could be recognized in the field. Some reports on weeds susceptible to the virus have been published; these are discussed in connection with our results (pp. 570–571.)

METHODS

Weeds grown from seeds were experimentally infected with the virus by mechanical inoculation using the carborundum method described by Rawlins and Tompkins (1936).[4] The virus was recovered from each species of weed and transferred to sugar beets by the same method.

WEED HOST RANGE AND SYMPTOMATOLOGY

Six species of weeds in three genera of the family Chenopodiaceae were experimentally infected by mechanical inoculation with the virus extract from mosaic-infected beets. Systemic infection occurred in all of the weeds from which the virus was recovered. The symptoms on the weeds experimentally infected with the virus are as follows.

Bractscale. The symptoms on bractscale, *Atriplex bractcosa*, are cessation of growth and a bending and curling of the apical shoot (plate 1, *B*) on infected plants. The young leaves are dwarfed, cupped outward, occasionally twisted along the midrib (plate 1, *C*) sometimes asymmetrical, mottled with small chlorotic spots; later the leaves become necrotic. Necrosis of the young leaves and death of the apical shoot occur within 3 weeks after inoculation.

Red Orache, or Redscale. The symptoms on red orache, *Atriplex rosea*, develop in essentially the same manner as those described on bractscale. All of the infected plants died within 3 weeks after inoculation.

[1] Received for publication December 21, 1946.

[2] Entomologist in the Experiment Station.

[3] Formerly graduate student in Entomology and Parasitology.

[4] See "Literature Cited" for citations, referred to in the text by author and date.

Spear Orache, or Spearscale. On spear orache, *Atriplex patula* var. *hastata,* small, chlorotic spots appear on the youngest leaves and these gradually enlarge on the somewhat older leaves (plate 2, *D*).

Lamb's-Quarters, or White Pigweed. The onset of mosaic symptoms on lamb's-quarters, *Chenopodium album,* is marked by dwarfed leaves with chlorotic spots and retarded growth of the apical shoots. The apical shoots are bent (plate 1, *F*, *G*), the youngest leaves curled, and cupped outward. Mottling develops on the youngest and older leaves (plate 1, *G*). Some leaves are not mottled but show numerous chlorotic rings surrounding green tissue (plate 1, *E*) ; later the rings become necrotic. Young leaves on the apical shoots become necrotic and dry (plate 1, *F*, *G*), and eventually the entire plants are killed.

This confirms the findings of Smith (1937), who recorded lamb's-quarters as a host plant of beet mosaic in England and described the symptoms on this weed.

Sowbane, or Nettle-Leaf Goosefoot. The first visible symptom of the disease on sowbane, *Chenopodium murale,* are cleared veins and veinlets (plate 2, *C*, lower row) followed by small, chlorotic areas on young leaves of the apical and axillary shoots (plate 2, *A*, *B*). Growth is retarded and the leaves become twisted, curled outward, and sometimes asymmetrical (plate 2, *A*, *B*). Necrosis of isolated spots or large chlorotic areas results in the death of the young leaves on both apical and axillary shoots, and necrotic streaks extend down the stem in the later stage of the disease (plate 2, *B*). Death of infected plants usually occurs within 4 weeks or less after inoculation.

Russian Thistle. The first reliable symptom on seedlings of Russian thistle, *Salsola kali* var. *tenuifolia,* was stunting of the apical and axillary shoots. Small, necrotic, sunken areas on the needlelike leaves, giving a beaded appearance, soon cause death and blackening of the shoots (plate 2, *E*). Necrotic streaks develop on the stem, and the infected seedlings die within 2 weeks after inoculation.

NONSUSCEPTIBLE WEEDS

The following weeds are nonsusceptible to beet mosaic. All inoculated weeds which failed to show symptoms of the disease were tested for the recovery of the virus.

Compositae, sunflower family: common sow thistle, *Sonchus oleraceus;* prickly sow thistle, *S. asper*

Cruciferae, mustard family: common yellow mustard, *Brassica campestris*

Malvaceae, mallow family: dwarf mallow, *Malva rotundifolia*

Plantaginaceae, plantago family: common plantain, *Plantago major*

Polygonaceae, buckwheat family: curly dock, *Rumex crispus;* fiddle dock, *R. pulcher;* sheep sorrel, *R. acetosella*

Solanaceae, nightshade family: black nightshade, *Solanum nigrum;* stramonium, *Datura stramonium.*

A number of investigators have reported the transmission of the beet-mosaic virus by means of aphids or mechanical inoculation to a number of weeds in many families contrary to the results of the present investigations. Novinenko (1930) states that *Aphis fabae* Scopoli was able to transmit the

beet-mosaic virus to *Amaranthanus retroflexus* (Amaranthaceae), *Chenopodium album* (Chenopodiaceae), and *Sonchus arvensis* (Solanaceae).

Our investigations failed to show that common sow thistle, *Sonchus oleraceus*, and prickly sow thistle, *S. asper* (Solanaceae), were susceptible to beet mosaic, nor could the virus be recovered from these weeds.

Verplancke (1933) reports that forty-five species of weeds in twenty-two families harbored the virus, but his investigations on the weed-host range were discredited by Quanjer and Roland (1936) and Quanjer (1936).

A number of species of aphids have been found in nature in the United States, on weeds susceptible to the virus. Essig (1926) reports that the bur clover or cowpea aphid, *Aphis medicaginus* Koch, was taken on lamb's-quarters, *Chenopodium album*. Gillette and Palmer (1931-1934) collected *A. medicaginus* and the green peach aphid, *Myzus persicae* (Sulzer) on lamb's-quarters and on Russian thistle, *Salsola kali* var. *tenuifolia*. *Aphis medicaginus* and *Myzus persicae* have been demonstrated to be vectors of the beet-mosaic virus to economic plants (unpublished data) and in all probability are vectors of the virus to susceptible weeds under natural conditions.

SUMMARY

Six species of weeds in three genera in the family Chenopodiaceae were experimentally infected by mechanical inoculation with the virus extract from sugar-beet mosaic. Systemic infection resulted in all of the weeds from which the virus was recovered. The species infected were as follows:

Bractscale, *Atriplex bracteosa*
Red orache, or redscale, *Atriplex rosea*
Spear orache, or spearscale, *Atriplex patula* var. *hastata*
Lamb's-quarters, or white pigweed, *Chenopodium album*
Sowbane, or nettle-leaf goosefoot, *Chenopodium murale*
Russian thistle, *Salsola kali* var. *tenuifolia*

Nine species of weeds in the families Compositae, Cruciferae, Malvaceae, Plantaginaceae, Polygonaceae, and Solanaceae were nonsusceptible to the virus.

LITERATURE CITED

ESSIG, E. O.
1926. Insects of western North America. 1035 p. The Macmillan Co., New York, N. Y. Reprinted 1934.

GILLETTE, C. P., and M. A. PALMER.
1931–1934. The Aphidae of Colorado. *Reprinted from:* Ann. Ent. Soc. Amer. **24**(4):827–934, **25**(2):369–496,**27**(2):133–255.

NOVINENKO, A. I.
1930. Insects as transmitters of mosaic diseases of the sugar beet. English summary p. 111. *In:* Muraviov, V. P. Mosaic diseases of the sugar beet. Plant Breeding Dept. of the Union Sugar Industry. U.S.S.R. 286 p. *Abstracted in:* Rev. Appl. Mycol. **11**:89–91.

QUANJER, H. M.
1936. Historique des rescherches sur la jaunisse et la mosaïque de la betterave. Inst. Belge pour l'Amélior. de la Betterave Pubs. **4**:23–33.

QUANJER, H. M., and G. ROLAND.
1936. De vergelingsziekte en de mozaiekziekte van de suiker–en voederbieten. The yellowing and mosaic diseases of sugar and fodder beet. I. (by Quanjer). Geschiedenis van het onderzoek over de vergelingsziekte en de mozaiekziekte van de beit. History of the investigation on the yellowing and mosaic diseases of beet. II. (by Roland). Onderzoek van de vergelingsziekte van de biet, met enkele opermerkingen over de mozaiekziekte. Investigations on the yellowing disease of beet with some observations on the mosaic disease. English summary by joint authors. Tijsch. Plziekt. **42**(3):45–70. *Abstracted in:* Rev. Appl. Mycol. **15**:548.

RAWLINS, T. E., and C. M. TOMPKINS.
1936. Studies on the effect of carborundum as an abrasive in plant virus inoculations. Phytopathology **26**(6):578–87.

SMITH, K. M.
1937. A textbook of plant virus diseases. 615 p. P. Blakiston's Son & Co., Inc., 1012 Walnut St., Philadelphia, Pa.

VERPLANCKE, G.
1933. Les viroses de la betterave. Sucr. Belge **53**:2–10, 22–32. *Abstracted in:* Rev. Appl. Mycol. **13**:210–11. 1934.

4½*m*-12,'47(A4262)

PLATES

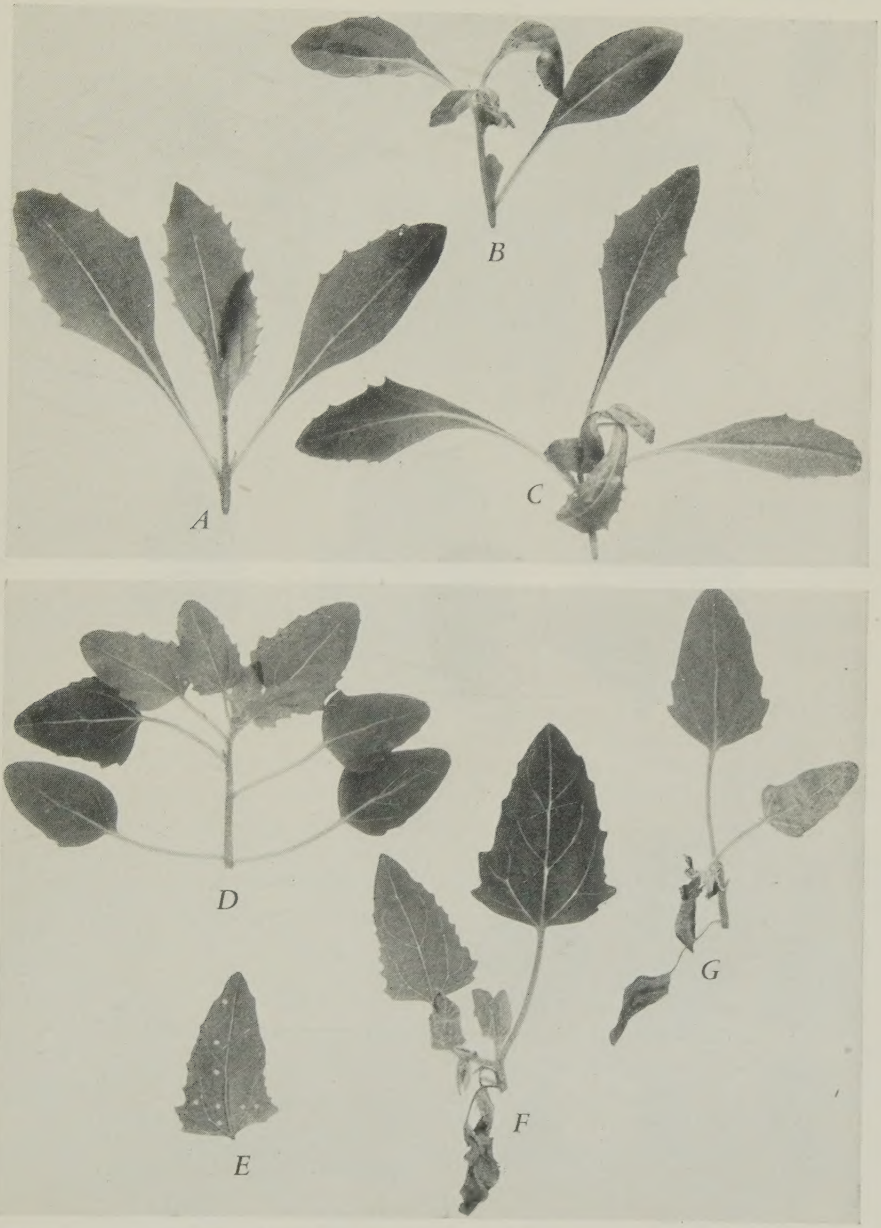
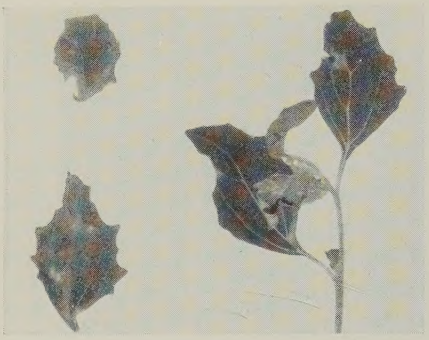


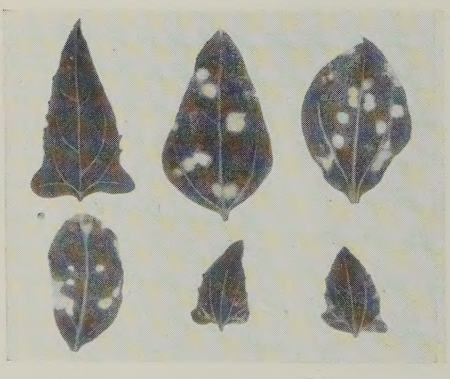
Plate 1.—*A–C*, Bractscale, *Atriplex bracteosa*: *A*, apical shoot from healthy check or control plant; *B*, *C*, apical shoots from plants experimentally infected with beet-mosaic virus, *B* showing bending and curling of dwarfed, youngest leaves, and *C* showing outward-cupped younger leaves and leaf twisted along the midrib. *D–G*, Lamb's-quarters, or white pigweed, *Chenopodium album*: *D*, apical shoot from check or control plant; *E–G*, leaves and shoots from plants experimentally infected with beet-mosaic virus, *E* showing chlorotic rings surrounding green tissue on young leaf, *F* showing bending of apical shoot, dwarfed youngest leaves, and dead leaves; and *G* showing necrotic and dead youngest leaf on apical shoot and mottling of older leaf.



A

B

C



D

E

Plate 2.—Weeds experimentally infected with beet-mosaic virus: *A–C*, Sowbane, or nettle-leaf goosefoot, *Chenopodium murale: A*, left, two leaves showing chlorotic spots; right, bending of apical shoot with outward-cupped and twisted, yellow-spotted leaf; *B*, bending of apical end of branch and axillary shoots with dwarfed, curled, or twisted leaves, chlorotic areas on older leaves, and other leaves necrotic and dead, and with black, necrotic streaks extending along the stems and axillary shoots; *C*, left, leaf from check or control plant, two others showing mottling and chlorotic spots. *D*, Spear orache or spear scale, *Atriplex patula* var. *hasta:* upper row, left, leaf from check or control plant, all others showing chlorotic spots. *E*, Russian thistle, *Salsola kali* var. *tenuifolia*, shoot showing necrotic, black, axillary leaves and necrotic streaks on the stem.